(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 255 249 A2**

(12) EUROPEAN PATENT APPLICATION

(21) Application number: 87305998.4

(22) Date of filing: 07.07.87

(51) Int. Cl.⁴: C12P 21/00 , A61K 39/395 , G01N 33/577 , G01N 33/68 , //G01N33/566,G01N33/574

(30) Priority: 07.07.86 US 882181
01.07.87 US 69412

(43) Date of publication of application:
03.02.88 Bulletin 88/05

(84) Designated Contracting States:
AT BE CH DE ES FR GB GR IT LI LU NL SE

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(54) Monoclonal antibodies to Fc receptors for immunoglobulin G on human mononuclear phagocytes; bifunctional antibodies; target specific effector cells; targeted macrophages; and immunoassays.

(57) A human Fc receptor-specific monoclonal antibody is disclosed together with its mode of preparation. Binding of the antibody to Fc receptor is not blocked by human immunoglobulin G. The antibody binds to the high affinity Fc receptor for IgG on human monocytes at a receptor binding site distinct from the ligand binding site for Fc.

A bifunctional antibody or a heteroantibody has an antigen binding region derived from an anti-Fc receptor antibody and an antigen binding region specific for a target epitope or cell; such antibody may target a macrophage when it is bound to surface Fc receptors of the macrophage.

A target-specific effector cell expresses receptor for the Fg portion of IgG, has one antigen binding region derived from an anti-Fc receptor antibody and another specific for a target cell, and the aforesaid bifunctional or hetero-antibody is bound to the Fc receptor of the effector cell; such effector cell can be used in the therapy of cancers, allergies, infectious and autoimmune diseases, and in immunoassays.

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"MONOCLONAL ANTIBODIES TO Fc RECEPTORS FOR IMMUNOGLOBULIN G ON HUMAN MONONUCLEAR PHAGOCYTES; BIFUNCTIONAL ANTIBODIES; TARGET SPECIFIC EFFECTOR CELLS; TARGETED MACROPHAGES; AND IMMUNOASSAYS"

The present invention relates to monoclonal antibodies to Fc receptors for immunoglobulin G on human mononuclear phagocytes; bifunctional antibodies; target specific effector cells; targeted macrophages; and immunoassays.

Several types of effector cells have surface receptors that bind the Fc portion of immunoglobulin (IgG). When such cells (monocytes, granulocytes, K cells, etc.) encounter target cells that have been opsonized with IgG antibodies, they form conjugates with the target cells. Subsequently, the effector cells either lyse or phagocytose the target cells, depending upon the effector cell type, the target cell type and the specific Fc receptor type involved.

Two distinct classes of IgG Fc receptors (FcR) have been identified on human monocytes and on the human monocytic cell line U937. Looney, R.J., et al., (1986) J. Immunol . 136:1641-1647. One is a 72kDa sialoglycoprotein (p72) with high affinity Ka=10^8-10^9 M-1) for monomeric human IgG1 and IgG3 and for murine subclasses IgG2a and IgG3. Alexander, M.D., et al. (1978)Immunol. 35:115-123; Anderson, C.L. and Abraham, G.N. (1980) J. Immunol. 125:2735-2741; Lubeck, M.D. et al. (1985) J. Immuno. 135: 1299-1304. The other receptor is a 40kDa molecule (p40) which shows relatively low affinity for monomeric IgG. Looney, et al., supra ; Jones, D.H., et al. (1985) J.Immunol. 135:33483353. P40 has been defined by its ability both to form rosettes with erythrocytes coated with murine IgG1 and to bind aggregated murine IgG2b at low ionic strength. In addition, a monoclonal antibody (IV3) has been prepared which binds to the 40 kDa receptor and inhibits ligand binding. See Looney, R.J., et al., supra. This receptor is present not only on mononuclear phagocytes but on human platelets, neutrophils and eosinophils. Rosenfeld, S.I., et al. (1985) J. Clin. Invest. 76:2317-2322.

These two Fc receptors on human monocytes have been shown to mediate anti-T3-induced human T cell mitogenesis by distinct subclasses of murine IgG. The 72kDa FcR mediates murine IgG2a anti-T3-induced stimulation whereas the 40 kDa FcR mediates murine IgGl anti-T3-induced T cell mitogenesis. See Looney et al ., supra. Based upon their distinctive affinities for murine IgG subclasses, p72 and p40 are thought to be the human homologues of murine macrophage FcRI and FcRII specific for murine IgG2a and IgG2b/1, respectively. Although not present on monocytes or U937 cells, a third class of IgG FcRs has been described on human neutrophils and null cells.

It has been demonstrated that target cell conjugation and lysis can also be induced by covalently cross-linked heteroantibody made up of both anti-Fc receptor antibody and antibody directed against a target cell epitope. When effector cells bind such heteroaggregates to their Fc receptor, they can specifically bind and lyse target cells which have not been opsonized, but which express the appropriate target antigen. Segal et al. have recently reported cytolysis of tumor cells by mouse monocytes with an attached heteroantibody which joins the Fc receptor of the monocyte on one end with tumor cell epitopes on the other end. The targeting of effector cells with conventional heteroantibodies, however, is likely to be only marginally effective in vivo because the binding of antibody to Fc receptors can be blocked by physiological concentrations of IgG.

Disclosure of the Invention

This invention pertains to monoclonal antibodies which are specific for the human high affinity Fc receptor for IgG, the p72 receptor, and which bind to the Fc receptor without being blocked by human IgG. The antibodies bind specifically, through their antigen combining region and independent of their Fc portion, to human receptor for the Fc portion of IgG. The antibodies bind to a site on the Fc receptor distinct from the binding site for the Fc region of IgG (ligand) and the antibodies are capable of binding a ligand-occupied receptor.

The anti-Fc receptor antibody of this invention can be made by monoclonal antibody producing techniques. Fc receptor protein can be obtained for immunization by preparing a cell fraction containing Fc receptor from a cell line that expresses Fc receptor (e.g. the U937 line, a human monocytic cell line that expresses Fc receptor for human IgG). The cells can be pretreated in culture with IFN-gamma to enhance the yield of Fc receptor protein. Fc receptor protein is purified by affinity purification from cell lysates. An

animal is immunized with the purified receptor protein and antibody-producing cells are harvested from the animal and fused with a myeloma cell or other immortalizing cell to produce hybridomas. The hybridomas are cloned and clones are selected for production of antibody to Fc receptor which is not blocked by human IgG.

The selection of antibody which binds to the Fc receptor through its antigen binding region (distinct from the Fc portion of the antibody) is complicated by the fact that the Fc portion of IgG of the animal species may bind human Fc receptor. For example, two of the four murine IgG subclasses -IgG2a and IgG3 - bind to the high affinity human Fc receptor via their Fc portion. In such instances selection can be facilitated as follows: After initial screening of hybridomas for production of Ig which binds the receptor, hybridomas which produce antibody of the subclass which is bound via its Fc region by the human Fc receptor, are eliminated from consideration. The remaining hybridomas are evaluated for production of antibody which binds Fc receptor independently of their Fc portion.

The anti-Fc receptor antibody of this invention can be used to produce target-specific effector cells for treatment of cancer, allergies, and infectious and autoimmune diseases. Antibody specific for a target cell (targeting antibody) can be linked to the Fc receptor of effector cell through the Fc-specific antibody of this invention. The linkage mediated by this anti-Fc receptor antibody is not disruptable by IgG because binding the receptor does not involve the Fc portion of the antibody.

For the purpose of targeting effector cells, a bifunctional antibody (used herein to mean a single antibody or antibody fragment with a dual binding specificity) or a heteroantibody (used herein to mean an aggregate of two or more antibodies (or antibody fragments) each antibody having a different specificity) can be produced. In general, the bifunctional antibody or heterantibody comprises:

a. at least one antigen binding region derived from an anti-Fc receptor antibody whose binding to human Fc receptor is not blocked by human immunoglobulin G; and

b. at least one antigen binding region specific for a target cell.

The binding of bifunctional or heteroantibody to the effector cell results in a targeted effector cell i.e., an effector cell with attached bifunctional or heteroantibody containing antigen binding regions which are specific for a desired target cell. The targeted effector cells can be used to bring about antibody dependent cell mediated cytolysis (ADCC) of the target cells in vivo.

The target cell can be a cancer cell or other cell whose elimination would be beneficial to the host, for example, an auto-antibody producing cell found in autoimmune diseases, or an IgE producing cell found in allergies. The target cell specificity of the bifunctional antibody or the hetero-antibody is derived from a targeting antibody i.e., an antibody specific for a target cell-associated or target cell-specific antigen. The use of the Fc specific antibody of this invention provides for attachment of the targeting antibody to monocyte effector cells by a linkage which is not disrupted by physiological levels of immunoglobulin G encountered in vivo. Thus, the targeted effector cells can be given in vivo without loss of effector cell specificity due to IgG competion for Fc receptor sites.

The anti-FcRI antibody of this invention has other therapuetic applications as well as several diagnostic applications. The antibody can be used as a targeting antibody to target FcRI-bearing cells. The antibody can also be used to induce capping and removal of Fc receptors on monocyte or other cells. Diagnostic applications of the antibodies include their use in assays for FcRI receptor levels and assays for substances that influence FcRI receptor levels.

The invention will now be described in more detail in the following description, which is given by way of example only, and which is to be read in conjunction with the accompanying drawings, in which:

Figure 1 shows SDS-PAGE of affinity adsorbed lysates of surface radioiodinated U937 cells.

Figure 2 shows SDS-PAGE analysis of affinity adsorption with ligand or with mab 32 after pre-clearing U937 lysates with ligand or with mab 32.

Figure 3 shows the results of isoelectric focussing of p72 purified either with ligand or with mab 32.

Figure 4 shows that human IgG does not interfere with the binding of Mab 32 to U937 cells, but blocks, almost completely, the binding of the mouse IgG2a myeloma UPC-10.

Figure 4b shows that human IgG does not interfer with the binding of Mab 32, 22, 44, 62 and 197 to U937 cells, but blocks almost completely the binding of mouse IgG2a UPC-10; and the increased binding of Mab 32, 22, 44, 62 and 197 to IFN-gamma treated U937 cells.

Figure 5 shows the fluorescence intensity of cells stained with mab 32.

Figure 6 shows the cytotoxicity of chicken red blood cells (cRBC) by IFN-gamma treated U937 cells mediated by the heteroantibody Mab32 x Fab anti-cRBC.

Figure 7 shows cytotoxicity of cRBC by interferon-gamma treated and untreated U937 cells.

Figure 8 shows cytotoxicity of chicken cRBC by interferon-gamma treated and untreated human peripheral blood monocytes.

Figure 9 shows the cytoxicity of cRBC by IFN-gamma treated U937 cells in the presence of the heteroantibody Mab 32 x Fab anti-cRBC and human IgG1.

Figure 10 shows cytotoxicity of cRBC by IFN-gamma treated and untreated human peripheral blood monocytes in the presence of the heteroantibody Mab 32 x Fab anti-cRBC and human IgG1.

Detailed Description of the Invention

The antibody of this invention binds the high affinity (p72) Fc receptor (FcRI) for human IgG without being blocked by human IgG. Preferred anti-FcRI receptor antibody has the following characteristics:

a. the antibody reacts specifically with the high affinity Fc receptor;

b. the antibody reacts with the receptor through its antigen combining region independent of its Fc portion;

c. the antibody reacts with an epitope of FcRI which is distinct from the Fc (or ligand binding) site of the receptor; and

d. the antibody binds ligand (Fc) occupied receptor.

The monoclonal anti-Fc receptor antibody of this invention can be produced by conventional monoclonal antibody methodology e.g., the standard somatic cell hybridization technique of Kohler and Milstein, Nature 256: 495 (1975). Although somatic cell hybridization procedures are preferred, in principle, other techniques for producing monoclonal antibody can by employed e.g., viral or oncogenic transformation of B lymphocytes.

Fc receptor for immunization of an animal can be prepared from lysates of human cells which express the receptor. A preferred receptor-bearing cell line is the human monocytic cell line U937; however, other monocytic cells such as HL-60 cells or freshly isolated monocytes can be used. Because interferon-gamma enhances Fc receptor expression, the cells can be cultured in the presence of interferon-gamma (e.g. 100 IU/ml) before receptor preparation to enhance the yield of receptor protein.

A partially purified preparation of the receptor can be made by lysing receptor-bearing cells and then purifying the receptor by immunoadsorbant chromatography. Cells can by lysed in a buffer containing a detergent such as NP40. The immunoadsorbent can be prepared by attaching human IgG to a water-insoluble material such as an activated Sepharose™ resin. The Sepharose resin with attached human IgG is poured into a column. The cell lysate is passed through the column under conditions which permit adsorbtion of the cellular Fc receptor protein by the IgG coupled to the resin. The adsorbed Fc receptor protein can be eluted with a mildly acidic elution buffer. The purified receptor can then be used for immunization of an animal to produce anti-receptor monoclonal antibody.

As an alternative to the use of partially purified receptor protein, whole FcRI-bearing cells can be used as immunogen. For example, whole interferon-gamma treated U937 cells can be used to elicit anti-FcRI antibody.

The preferred animal system for preparing hybridomas is the murine system. Hybridoma production in the mouse is a very well-established procedure. Immunization protocols and techniques for isolation of immunized splenocytes for fusion are well known in the art. Fusion partners (e.g., murine myeloma cells) and fusion procedures are also well-known.

Selection of murine hybridomas producing antibody against the FcRI for IgG of human monocytes, however, is complicated by the fact that two subclasses of murine IgG - the IgG2a and IgG3 subclasses - are ligands capable of binding with high affinity to this receptor. Thus, assays for monoclonal antibody capable of binding to the receptor would register as positive all murine antibodies of these two subclasses. This obstacle can be avoided by first screening hybrid cells for production of antibody reactive with the cell line which was the source of Fc receptor, then elimination hybrid cells which produce IgG2a and IgG3 antibodies and finally, evaluating remaining hybridomas for production of antibody against high affinity receptor. This strategy is further detailed in the exemplification below.

Employing the methodology described, five murine monoclonal anti-FcRI antibodies were prepared. The antibodies are designated mab 22, mab 32, mab 44, mab 62 and mab 197. Each of the antibodies exhibit the preferred characteristics set forth above.

The anti-Fc receptor antibody of this invention can be used to produce target-specific effector cells i.e. effector cells which are capable of recognizing and binding to a target cell and exerting their effector function. It provides a means for attaching to an effector cell an antibody or antibody-binding fragment directed against a target cell. The attachment is not disruptable by physiological concentrations of IgG because the anti-Fc antibody which mediates the attachment binds the receptor through its antigen-binding region. Effector cells, such as macrophages, targeted in this way can be employed to bring about antibody-dependent cell-mediated killing of target cells.

To target effector cells, bifuctional antibodies or heteroantibodies are employed. These antibodies have dual antigen binding specificity -one specificity for the Fc receptor (preferably the high affinity Fc receptor) and one specificity for an epitope of the target cell. The Fc receptor specificity mediates linkage to the effector cell through a known cytotoxic trigger molecule. The target cell specificity provides for recognition and binding to the target cell.

Bifunctional antibodies are single, divalent antibodies which have two different antigen binding sites. Bifunctional antibodies for targeting have one binding site for Fc receptor and one binding site for a target cell epitope.

Hetereoantibodies are two or more antibodies or antibody binding fragments (Fab) linked together, each antibody or fragment having a different specificity. Heteroantibodies for targeting comprise an antibody (or antigen binding fragment specific for Fc receptor, coupled to an antibody (or antigen binding fragment thereof) specific for a target cell epitope.

Bifunctional antibodies can be produced by chemical techniques (see e.g., D. M. Kranz et al., Proc. Natl. Acad. Sci. USA 78,5807 (1981)) by "polydoma" techniques (See U.S. Patent 4,474,893, to Reading) or by recombinant DNA techniques. heteroantibodies can be prepared by conjugating Fc receptor antibody with antibody specific for an epitope of a target cell. A variety of coupling or cross-linking agents can be used to conjugate the antibodies. Examples are protein A, carboiimide, and N-succinimidyl-3-(2-pyridyldithio) proplonate (SPDP). SPDP is the preferred agent; procedures for crosslinking antibodies with this agent are known in the art. See e.g., Karpovsky et al., (1984) J. Exp. Med. 160:1686; Liu, M.A. et al., (1985) Proc. Natl. Acad. Sci USA 82:8648.

Target cells are cells whose elimination would be beneficial to the host. One important type of cell is a tumor cell. Effector cells can be targeted with bifunctional or heteroantibody having specificity for FcRI and specificity for a tumor associated or tumor specific antigen.

Antibodies with a desired tumor specificity for production of bifunctional antibody or hetero-antibody can be produced or can be selected from available sources. Monoclonal antibodies against tumor-associated antigens can be made by the methods of Koprowski et al., U.S. Patent 4,172,124. Many suitable anti-cancer antibodies are presently available.

Specific anti-tumor antibodies would include, but not be limited to:

Antibody	Specificity
AML-2-23, PM-81, PMN-6, PMN-19	Myeloid Leukemia
SCCL-1, SCCL-175	Small Cell Carcinoma of the Lung
OC1-25, OVCT-3	Ovarian Carcinoma
COL-1, COL-2, COL-3, ... COL-13	Colon Carcinoma

In addition to tumor cells, the effector cell can be targeted against auto-antibody producing lymphocyte for treatment of autoimmune disease or an IgE-producing lymphocyte for treatment of allergy. The target can also be microorganism (bacterium or virus) or a soluble antigen (such as rheumatoid factor or other auto-antibodies).

Effector cells for targeting are human leukocytes, preferably macrophages. Other cells would include monocytes, IFN-gamma activated neutrophils, and possibly IFN-gamma activated natural killer (NK) cells and eosinophils. Macrophages can be treated with IFN-gamma before targeting, to increase the number of Fc receptors for attachment of the targeting antibody or heteroantibody. The effector cells may also be activated before targeting by other cytokines such as tumor necrosis factor, lymphotoxin, colony stimulating factor, and interleukin-2. If desired, effector cells for targeting can be obtained from the host to be treated.

The targeted effector cells can be administered as a suspension of cells in a physiologically acceptable solution. The number of cells administered can be in the order of 10^8-10^9 but will vary depending on the therapeutic purpose. In general, the amount will be sufficient to obtain localization at the target cell and to effect target cell killing by antibody dependent mediated cytolysis (ADCC). Routes of administration can also vary. In tumor therapy, for instance, depending upon the localization of a tumor, the targeted effector cells could be administered intravenously, or directly into tumor sites; as for example, directly into the peritoneal cavity in the case of ovarian carcinoma.

Therapy with targeted effector cells can be performed in conjunction with other techniques for removal of targeted cells. For example, anti-tumor therapy with effector cells armed with FcRI/anti-tumor antibody can be used in conjunction with surgery, chemotherapy or radiotherapy. Additionally, combination immunotherapy may be used to direct two distinct cytotoxic effector populations toward tumor cell rejection. For example, anti-tumor antibodies linked to anti-T3 that will trigger cytolytic T lymphocytes to lyse tumor cells may be used in conjunction with anti-RcRI antitumor hetero-antibodies. Protocols based on these concepts may be especially effective in removing residual tumor cells in patients induced into remission by chemotherapy and irradiation.

The anti-Fc receptor antibody of this invention has additional utility in therapy and diagnosis. The Fc receptor antibody itself can be a targeting antibody (i.e., to target for cells bearing FcRI receptor). The antibody can be used to target lipid vesicles containing anticancer drugs for treatment of certain hematological cancers (e.g. acute myeloid leukemia), or to target lipid vesicles containing factors (such as gamma-IFN) which activate monocytes. The antibody, if of the appropriate murine IgG subclass (e.g., IgG2a), can be used directly in vivo to eliminate Fc receptor bearing cells (e.g., myeloid leukemia cells) via natural complement or ADCC mechanisms.

The antibody can be employed to modulate Fc receptor levels on monocytic cells. For example, in auto-immune diseases (such as rheumatoid arthritis) the antibody can be administeredin a form that induces "capping" and elimination of Fc receptors on the cell surface. The reduction of Fc receptors can interfere with monocyte clearance of antibody coated self-cells in patients. Mixtures of anti-Fc receptors can also be used for this purpose.

Diagnostic applications of the anti-FcR antibody of the invention can be based on the use of the antibody to quantify the distribution or number of Fc receptors on cells. The antibody can be employed in assays for agents which influence receptor expression (e.g., Interferon-gamma, which enhances Fc receptor expression). For example, in an assay for interferon-gamma the anti-FcRI antibody labeled (radioisotopically, enzymatically or fluorescently) can be used to quantify FcRI levels on cells exposed to a test sample. Receptor levels will be related to the amount of interferoon-gamma in the sample.

The antibody can also be used to subclassify patients with rheumatologic disorders which are related to Fc receptor levels or to the ability of a patient's cells to respond to interferon by enhanced expansion of Fc recptors.

Based on the increased expression of FcRI on IFN-gamma plus dexamethasone-treated monocytes it is anticipated that anti-FcRI monoclonal antibodies will be excellent markers of inflammatory macrophages. It is possible that the accumulation and activation of mononuclear phagocytes at sites of inflammation (including but not limited to infectious foci, septic arthritis, atherosclerotic plaques) can be detected by radio-imaging using radiolabelled antibodies to the FcRI on such cells.

The invention is illustrated further by the following non-limitative exemplification:

Exemplification

Materials and Methods

Chemical and Reagents

Cytochrome c Type VI, superoxide dismutase, pepstatin, chymostatin, leupeptin, antipain, rabbit muscle actin and phenylmethylsulfonylfluoride (PMSF) were purchased from Sigma Chemical Co., St. Louis, MO; Dextran T500, Ficoll-Hypaque, Sepharose 4B, CNBr-activated Sepharose, Protein A-Sepharose CL-4B from Pharmacia Fine Chemicals, Piscataway, NJ; tetanus toxin, octyl-b-D-glucopyranoside (octyl-glucoside) and papain from Calbiochem, La Jolla, CA; human anti-tetanus toxin antibody (HyperTetTM) from Cutter Laboratories, Berkeley, CA; chloroglycouril from Pierce Chemical Co., Rockford, IL; carrier-free I^{125} - (IMS.300) from Amersham, Arlington Heights, IL; cytochalasin B from Aldrich Chemical Co., Milwaukee, WI; goat F(ab')2 antimurine Ig (anti-mIg), both fluorescein isothiocyanate-conjugated (FITC) and unconjugated,